THE CLINICAL FEATURES OF C9ORF72 AMYOTROPHIC LATERAL SCLEROSIS IN A FINNISH COHORT

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C9orf72-geenin toistojaksopidentymä on yleisin amyotrofista lateraaliskleroosia (ALS) ja frontotemporaalidementiaa (FTD) aiheuttava geenimutaatio. Tämän syventävän työn tarkoitus on kartoittaa C9orf72-geenimutaatioon liittyvän amyotrofisen lateraaliskleroosin taudinkuvaa suomalaisessa väestössä.

Tutkimusaineisto koostui 37:sta C9orf72-geenin osalta positiiviseksi todetusta ALS-potilaasta. Tutkimusjoukon potilasteksteistä kirjattiin taudin alkamistyyppi ja -aika, taudin kesto, ylemmän ja alemman motoneuronin oireet, dementia ja muistioireet, parkinsonismi, psykoosisairaudet sekä sukutausta. Koko potilasryhmän lisäksi näitä muuttujia tarkasteltiin tilastollisesti myös sukupuolen ja taudin alkamistavan sekä sukutaustan mukaan.

Potilaiden sairastumisikä oli keskimäärin 60,0 vuotta (vaihteluväli 33,3–80,8). 37,5 %:lla oli bulbaarisia oireita taudin alkaessa. Taudin kesto oli keskimäärin 39,9 kuukautta (vaihteluväli 10,0–249,0). Taudin keston pituudessa ei ollut eroa sukupuolten välillä, mutta miehillä oli selkeästi suurempi varianssi kuin naispotilailla (p=0,017). 37,8 %:lla potilaista oli lisäksi FTD ja yhteensä 59,5 %:lla joko dementia tai kognitiivisten kykyjen laskua. Miehillä FTD oli yleisempi (55,0 %) kuin naisilla (17,6 %) (p=0,04). Kolmella potilaalla taudin kesto oli huomattavan pitkä (110,0–249,0 kuukautta). Yhdellä potilaalla taudin alkamistapa oli käsiin rajoittuva ns. flail arm, ja yhdellä spastinen parapareesi. Ylemmän motoneuronin oireista huomattavasti yleisin oli refleksien vilkkaus.

Tutkimus vahvistaa aiempia käsityksiä C9orf72-geeniin liittyvän ALS:n ilmiasun monimuotoisuudesta, johon sisältyy myös hyvin pitkä taudin kesto osalla potilaista. Mahdollisia sukupuolieroja taudin fenotyypin suhteen on syytä tutkia jatkossa.

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1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease which leads to muscle weakness, atrophy, spasticity and eventually death from respiratory failure, usually within 3–5 years from symptom onset. According to revised El Escorial criteria, ALS requires 1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathological examination, 2) evidence of upper motor neuron (UMN) degeneration by clinical examination, 3) progressive spread of symptoms or signs within anatomical regions or to other regions, and 4) no evidence of other disease processes that might explain these symptoms and signs (1, 2).

By its very definition, ALS is thought to affect mostly the lower and upper motor neurons. However, it is also related to cognitive impairment in up to 50% and frontotemporal dementia (FTD) in 13.8% of patients (3, 4). Similarly, a subset of FTD patients presents with motor neuron symptoms, and FTD and ALS are emerging as different manifestations of the same disease spectrum (5–10).

1.1 Genetic background of ALS

Most cases of ALS are sporadic (SALS), but a minority are familial (FALS) with both autosomal dominant and recessive inheritance patterns. The rate of FALS has been estimated at around 5–10 %, but the true rate is most likely higher and even as high as 16–20% (11–13). Even in sporadic ALS, twin studies suggest that heritability would explain 61% of disease development (14). Sporadic and familial ALS do not differ in their clinical picture, and are sometimes hard to differentiate due to uncertainty in family history (15).

Several genes have been implicated in the pathology of ALS. The most common autosomal dominant gene mutation is the GGGGCC hexanucleotide repeat in C9orf72, which was discovered in 2011 (16, 17). The other common dominant mutations include SOD1, TARDBP, and FUS. In Europe, C9orf72 accounts for 29.3–38.2% of FALS and ~5% of SALS (18).

According to a recent meta-analysis by Zou et al (2017), C9orf72 is less common in Asia than Europe, with a frequency of only 0.3–6.3% in FALS. Interestingly, the authors also discovered co-occurrence of the four most common mutations in 0.4% in FALS cases, which implies at least in part oligogenic pathology for ALS (18).

1.2 ALS in Finland

In Finland, the frequency of C9orf72-related ALS is higher than elsewhere, accounting for 46.0% of FALS patients and as much as 21.1% of SALS patients. In addition, SOD-1 mutation is particularly common in Finland, accounting for 42.9% of FALS and 11.3% of SALS. (15) Nearly all SOD-1 mutations are dominantly inherited, but the most common type in Finland, familial SOD-1 D90A, has a recessive inheritance pattern. The familial recessive SOD-1 D90A ALS has a markedly slow disease progression that usually starts from lower limbs with predominantly UMN signs. The average disease duration is over ten years and many live past two decades after disease onset. (19, 20) The recessive D90A SOD-1 allele is found mostly in Scandinavian countries and may be tightly linked with a protective genetic factor (10, 21).

Considering how common both C9orf72 and SOD-1 are in Finland, it is not a surprise that the incidence of ALS in Finland has been estimated at 2.4 per 100,000 person-years, which is one of the highest in the world (22). There may be more factors affecting the incidence rate, but the impact of these two mutations in the population is indisputable. Together, C9orf72 and SOD-1 mutations account for 87% of FALS in Finland (17).

1.3 C9orf72-related ALS

C9orf72 mutation causes both ALS and FTD. It is not yet clear why it presents as FTD in some patients and ALS, or a combination of both, in others (6). The exact pathological mechanism is still unclear, but accumulation of nuclear RNA foci, TDP-43 pathology and p62- and ubiquitin-positive dipeptide repeat protein inclusions are implicated (8). While the normal repeat length is between two and ten units for 90% of the population, it is still not known how many GGGGCC hexanucleotide repeats are required to induce disease (23). Initially, C9orf72 mutation was assumed fully penetrant, but there is now evidence of

incomplete penetrance (24, 25).

The clinical characteristics of the C9orf72-mutated ALS (C9-ALS) phenotype have been depicted in several cohort studies. C9-ALS is correlated with an earlier age at onset and shorter disease duration compared with non-C9 ALS (4, 18–22). Some studies have reported bulbar onset to be more common with C9orf72 (20, 23). Dementia has typically been present in a much higher proportion of patients compared with other ALS patients, most notably behavioral-variant FTD but also early-onset Alzheimer's disease (24, 25). Of note is also the observation that a high rate of psychosis and hallucinations, as well as parkinsonism have been reported in association with C9orf72 (12, 34). A recent study found a gender effect, with C9-ALS males with spinal onset having significantly lower survival compared with other ALS patients with spinal onset (35). There is also evidence of genetic anticipation, with mutation carriers in successive generations having earlier ages at onset (20, 28).

1.4 Purpose of the study

At this point, it is clear that C9-ALS seems to have high variability in its phenotype, and most likely several factors modifying its disease risk and disease course. The aim of this study is to describe the phenotype of C9-ALS in a Finnish cohort and report patient cases with atypical phenotypes.

2. METHODS

2.1 Data collection

All patients identified as C9orf72-positive at the department of Neurology in Tampere University Hospital before October 2017 were included in our study (n=30). In addition, seven cases from Seinäjoki Central Hospital, three cases from Vaasa Central Hospital and one

case from Turku University Hospital were included. All genetic investigations of the patients were performed in Tampere University Hospital.

The medical records of these patients were systematically reviewed to obtain details of disease phenotype including gender, age of onset, age at diagnosis, disease onset type (spinal, bulbar, or multifocal), disease duration, history of memory or cognitive function, parkinsonism, psychosis, upper motor neuron (UMN) symptoms and lower motor neuron (LMN) symptoms. Recorded upper motor neuron symptoms included spasticity, Babinski reflex, and hyperreflexia. Recorded lower motor neuron symptoms included muscle atrophy, fasciculations and an abnormal electroneuromyography (ENMG) finding. Patients who did not have a diagnosis of clinically definite, probable or possible ALS according to El Escorial revised criteria were left out of the study (n=6) (1, 2). These excluded cases presented only with symptoms of FTD and no significant motor neuron symptoms. In addition, one case was left out due to insufficient information. All patients included in the study had signed a written form of consent.

2.2 Statistical analysis

Collected data was analysed using IBM SPSS Statistics 23.0. Indiscrete variables such as age at onset, age at death and disease duration were described using median, mean, range and standard deviation and compared in subgroups based on gender, disease onset type, and family history using either 2-sample t-test or Mann-Whitney U-test. Variances were compared with Levene's test. Discrete variables (rate of dementia, psychosis, LMN and UMN symptoms) were described using frequency tables and compared in subgroups of gender and disease onset type with χ-square test or Fisher's exact test when applicable. A significance level of <0.05 was used for all statistical tests.

3. RESULTS

The clinical phenotype of 37 C9-ALS patients was analysed. Twenty of these patients were male and 17 female. By the end of our study period, eight patients were still alive without tracheostomy, and one patient with tracheostomy. One patient had died from myocardial infarction and 27 patients from ALS.

3.1 Age of onset

The mean age of onset was 60.0 years (SD 9.8 years), ranging from 33.3 to 80.8 years. 37.8% of patients had a bulbar or multifocal onset (24.0% and 13.5%, respectively). Spinal onset tended to have an earlier age at onset than bulbar onset (58.6 versus 62.3 years). However the difference was not statistically significant (p=0.25). There was no difference in disease onset type between sexes.

	Number of patients	**Mean age at onset, yrs**	**Mean age at diagnosis, yrs**	**Median disease duration, yrs**	**Median disease duration, deceased, yrs**	**Mean age at death, yrs**	**FTD, number/%**
All patients	37	60.0	62.3	3.3	3.0	65.2	14/37.8
Male	20 (54.1%)	58.8	61.5	3.2	3.2	63.4	11/55.0
Female	17 (45.9%)	61.4	63.2	3.3	2.9	67.8	3/17.6
Spinal onset	23 (62.1%)	58.6	61.6	3.7	3.6	64.6	9/39.1
Bulbar or multifocal onset	13 (37.8%)	62.3	63.2	2.5	2.1	65.7	5/35.7

Table 1. Characteristics of the cohort.

3.2 Disease duration

There was considerable variation in disease duration, which was defined by the time between symptom onset as reported by patients and the end of surveillance (time of death or tracheostomy, or October 2017). Disease duration was analysed in all patients, and separately including only those who had died from ALS. The range of disease duration varied from 10.0 months to 249.0 months (20.7 years). Median for disease duration was 39.9 months in all patients, and 35.8 months in deceased. There was no statistically significant difference between males and females. However, male patients had more variance in disease duration than female patients (p=0.017), and this seemed to be driven by higher variance in specifically spinal-onset males compared with all other patients (p=0.015) (See Fig. 1 and Table 2). Bulbar or multifocal onset group had a significantly shorter disease duration than spinal-onset group, among all patients (p=0.007) and among the patients who had died from ALS (p=0.006). We also compared spinal-onset males (n=14) and spinal-onset females (n=9) separately, but found no significant difference. More details of the clinical characteristics can be seen in Tables 1 and 2.

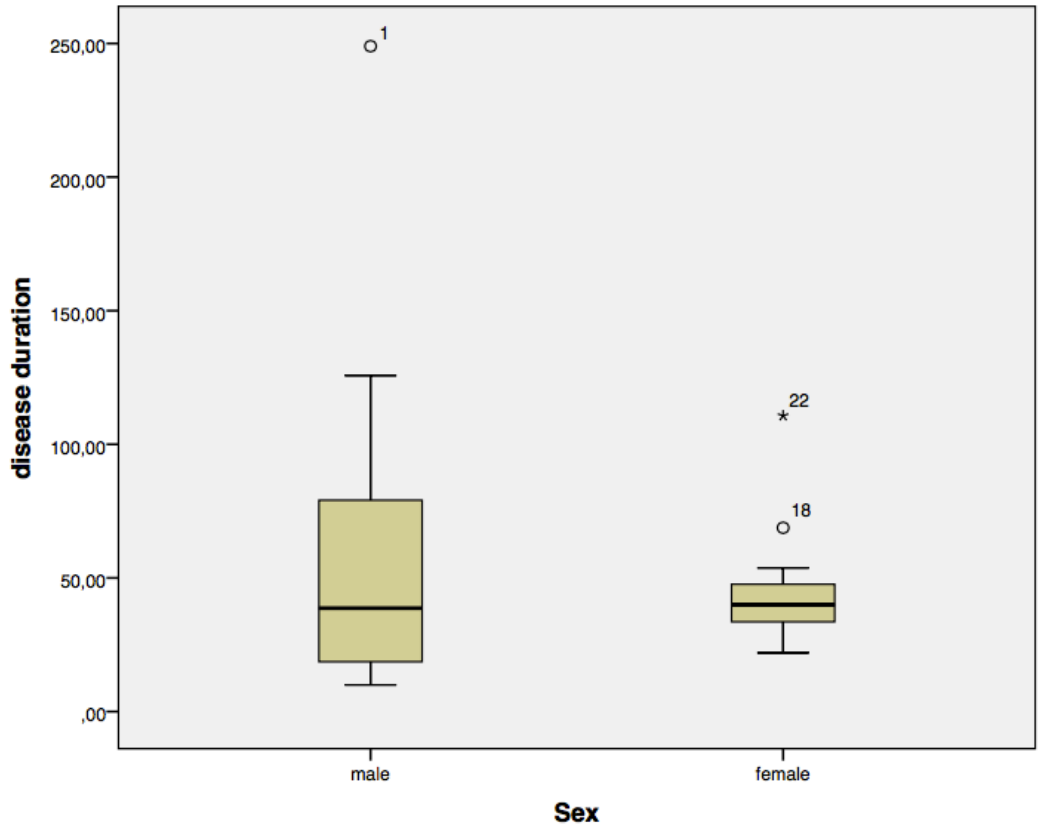

Figure 1. Disease duration (months) in male and female patients.

	Mean disease duration, months	Median disease duration, months	SD, months	Range, months	Difference in disease duration (Md) between sexes, p
Females	43.8	39.6	20.6	22.0–110.7	0.798
Males	55.1	38.6	55.7	10.0–249.0	
Spinal-onset females	47.9	39.9	25.0	25.9–110.7	0.403
Spinal-onset males	70.5	59.9	60.3	14.7–249.0	

Table 2. Disease duration in male and female patients and spinal-onset males and females.

3.3 Motor neuron symptoms

97.3% of patients had signs of lower motor neuron involvement. All symptoms of lower motor neuron involvement were commonly presented in our patients: 89.2% had signs of muscle atrophy, 78.4% had fasciculations and 73.0% had ENMG that filled diagnostic criteria for lower motor neuron signs of ALS (2). There was no significant difference between males and females, or between disease onset type. More details can be seen in Table 3a.

91.9% of patients had signs of upper motor neuron involvement. Hyperreflexia was the most common sign of upper motor neuron involvement and present in 78.4% of patients. Spasticity and an abnormal Babinski reflex were less common, presenting in 32.4% and 24.3% of patients, respectively. Spasticity seemed more common in spinal onset (43.5%) than in bulbar or multifocal onset (14.3%), but the difference was not statistically significant (p=0.14). More details can be seen in Table 3b.

	LMN involvement, %	Muscle atrophy, %	Fasciculations, %	ENMG diagnostic (abnormal), %	Muscle biopsy taken, %
All	97.3	89.2	78.4	73.0 (83.8)	32.4
Male	100.0	90.0	85.0	80.0 (85.0)	35.0
Female	94.1	88.2	70.6	64.7 (82.3)	29.4
Spinal onset	100.0	95.7	73.9	78.3 (91.3)	39.1
Bulbar or multifocal onset	92.9	78.6	85.7	64.3 (71.4)	21.4

Table 3a. Lower motor neuron symptoms in C9-ALS patients

	UMN involve-ment, %	Hyper-reflexia, %	Spasticity, %	Babinski positive (positive or indifferent), %	UMN excl. reflexes[1], %	UMN excl. reflexes[2], %
All	91.9	78.4	32.4	24.3 (51.3)	54.1	40.5
Male	90.0	75.0	35.0	25.0 (50.0)	55.0	45.0
Female	94.1	82.4	29.4	23.5 (52.9)	52.9	35.3
Spinal onset	87.0	82.6	43.5	30.4 (56.5)	60.9	52.2
Bulbar or multifocal onset	100.0	71.4	14.3	14.3 (42.9)	42.9	21.4

Table 3b. Upper motor neuron symptoms in C9-ALS patients.
[1] Rate of UMN pathology, excluding hyperreflexia.
[2] Rate of UMN pathology, excluding hyperreflexia and indifferent Babinski.

3.4 Dementia, cognition and neuropsychiatric disorders

As many as 59.5% of patients had either a diagnosis of dementia, or their medical records had noted issues with memory or cognitive decline. 37.8% had a diagnosis of FTD and 5.4%

Alzheimer's disease. Three cases had suffered from psychosis at some point in their lives, and one case from paranoia. One patient had been diagnosed with parkinsonism.

A gender effect was observed regarding cognitive symptoms, with the rate of FTD being significantly higher in male (55.0%) than in female patients (17.6%) (p=0.02). When considering all cognitive decline, the rate in male patients was also significantly higher than in female patients (75.0% versus 41.2%, p=0.04). Interestingly enough, all cases with psychosis also happened to be male (n=3). There was no difference in memory symptoms or FTD based on disease onset type.

3.5 Family history

75.7% of our patients had a positive family history of at least one family member affected by ALS or FTD. Age at onset, disease duration and age at the end of surveillance were compared in subgroups of positive and negative or unknown family history, but there were no statistically significant differences. However, the mean age of onset for patients with positive family history was lower than that of negative or unknown family history (58.3 versus 62.7 years), but this was not statistically significant (p=0.182).

3.6 Atypical patient cases

Three patients in the cohort had unusually long survival with disease duration extending over 100 months. All three were spinal-onset. Two were male and one female.

Patient 1 was a spinal-onset male with a positive family history (father and father's aunt having suffered from ALS). Symptoms started at age 34 from the left lower limb, and persisted in only lower limbs for the first six–seven years, after which symptoms spread to the upper limbs. The patient expressed both UMN symptoms (including spasticity, hyperreflexia, and indifferent Babinski sign), as well as LMN signs (muscle atrophy, fasciculations, diagnostic ENMG). The disease progression remained slow for over 15 years before rapidly increasing. The patient was then restricted to a wheelchair and his speech began to slur. He died from ALS at age 54, with an estimated disease duration of 20.8 years.

Patient 2 was a spinal-onset male with no known family history. His disease onset started from upper limbs at age 62. He showed predominantly LMN signs, as well as hyperreflexia as the only UMN sign. His muscle atrophy progressed very slowly, with disease duration 125 months (10.6 years) at the time of research. He is still alive at the time of reporting.

Patient 3 was a spinal-onset female whose mother had an undiagnosed motor neuron disease. Her symptoms began at 51 years as difficulty of walking, and remained isolated in just one leg and later in both lower limbs. Later on, she also developed mild signs of ALS in her upper limbs. Her symptoms have been UMN-dominant (spasticity, hyperreflexia, positive Babinski sign) with muscle atrophy as the only LMN sign. Her disease duration is 110 months (9.2 years) at the time of research and she is still alive at the time of reporting.

Furthermore, one spinal-onset male patient presented with a flail arm phenotype, with LMN symptoms and hyperreflexia. He died of traumatic suffocation 18 months after the onset of his symptoms. Another spinal-onset male patient presented with spastic paraplegia at the start of his motor neuron disease with mostly UMN symptoms, which later developed into classic ALS with both LMN and UMN symptoms. His disease duration was 41 months.

4. DISCUSSION

Our cohort of C9orf72-positive ALS patients showed considerable heterogeneity in their clinical presentation. Age of onset varied as much as 47.5 years (range 33.3–80.8 years) and disease duration 19.9 years (range 10.0–248.0 months). Our study population also included a number of cases with exceptionally slow disease progression and the clinical presentation being limited to just one or two limbs for a considerable time, reminiscent of recessive D90A SOD1 -linked ALS phenotype (20). All of these aforementioned findings reinforce that C9-ALS can present with any phenotype of ALS.

4.1 Disease onset and survival

Studies have reported an earlier age of onset and shorter survival for ALS in C9orf72-mutation carriers compared with non-carriers (4, 18–22). Our study did not have a control group of non-carriers, so we cannot compare our findings regarding differences with non-C9 ALS. In relation to C9-ALS, the mean age of onset (60.0 years) was in the same range or slightly higher as in other cohort studies, where the mean age of onset has varied between 54.5–59.3 years (4, 18–22). Disease duration (median 39.9 months, range 0.83–20.8 years) was somewhat longer in our cohort than in earlier reported studies (median 20–34.4 months, range 0.45–13 years) (6, 27–31). The difference may be in part due to the limitations of a fairly small cohort size, but it may also imply that disease progression in C9-ALS may be slightly slower in the Finnish population, possibly due to other disease-modifying genetic or environmental factors.

What was striking in our cohort were the few cases with extremely slow progression and long survival. Earlier cohort studies have also reported individual cases with exceptionally long tracheostomy-free survival: in a Dutch cohort study, Van Rheenen et al (2012) reported of one C9-ALS patient with 13 year-survival and also of one C9orf72-positive progressive muscular atrophy (PMA) patient with a survival of 21 years, who was still alive at the end of a study. Likewise, Gijselinck et al (2012) in a Flemish-Belgian cohort study reported of one case with 10-year survival. It is possible that these cases with slow progression could have a protective genetic factor (or factors) modifying disease course.

We compared disease course of C9-ALS in subgroups of patients based on gender and disease onset type, as well as family history. Bulbar symptoms were found in 37.8% of our patients at disease onset, in keeping with reports of 33–43.5% bulbar onset rate in C9-ALS patients (26–31). There were no statistically significant differences in disease onset type, age of onset or disease duration between sexes or family history. Spinal onset had a significantly longer disease duration than bulbar or multifocal onsets, which is a well-recognised phenomenon in ALS (37). Of interest is also the fact that age at onset was lower in patients with a positive family history, even though the difference did not reach statistical significance. However, this trend would be in keeping with findings of genetic anticipation in C9-ALS affecting age at onset in successive generations (28, 36).

A recent study by Rooney et al (2017) combined data from three separate ALS registers and found an intriguing gender effect, with C9-ALS spinal-onset males having shorter survival than other spinal-onset ALS patients. We did not find a difference in prognosis of spinal-onset males compared with spinal-onset females, although it may have been due to our study population being much smaller. Interestingly, we did find that spinal-onset males had significantly more variance in disease duration than other patients, and male patients more than female patients. The difference between sexes seemed to be mostly driven by spinal-onset males. This would support the previously noted gender effect in spinal-onset males affecting survival and disease course, even though our study did not perceive an effect on survival. How exactly this gender effect affects disease course, and what mechanism is behind it, are fascinating topics that warrant further research.

4.2 Motor neuron symptoms

C9-ALS patients have been found to present with typical motor features of ALS, usually having both UMN and LMN symptoms (6, 31). Correspondingly, our patients displayed a high frequency of both UMN symptoms (91.9%) and LMN symptoms (97.3%). However, upper motor neuron signs excluding hyperreflexia were seen in only 54.1% of patients, and similar rates were depicted in an earlier study (31). It seems that upper motor neuron symptoms apart from reflex pathology are difficult to discern in C9-ALS patients. This feature should be taken into account by clinicians in their clinical work when suspecting C9-ALS. The reason behind this remains unclear, but one possibility is that generalized muscle atrophy that is prevalent in C9-ALS patients could mask signs of upper motor neuron damage.

4.3 Dementia, family history and study limitations

The frequency of FTD and cognitive symptoms was much higher in our cohort (59.5%) than other studies with C9-ALS, where the frequency has been 31.8–50% (26, 28, 29, 31). Even though the rate of FTD and cognitive decline have been reported higher in C9-ALS, our result was most likely affected by the fact that C9orf72 gene test was taken only when clinicians

asked for it specifically, instead of routinely testing all ALS patients. Thus, a suspicion of C9-ALS has most likely risen more easily when motor neuron symptoms were combined with dementia or cognitive decline, and resulted in our study population having a skewed prevalence of memory symptoms. In the same vein, the very high frequency of a positive family background (75.7%) in our cohort has most likely been affected in a similar fashion, as the C9orf72 gene is known for being the most common cause for familial ALS.

An interesting gender effect was observed with FTD and cognitive decline being significantly more common in male than female patients. We could not find a satisfactory explanation for this finding, although not all confounding factors, such as alcohol use, have been accounted for. One drawback of our study that could affect this finding is the fact that dementia and cognitive decline were not systematically reviewed in each patient, and thus some cases are likely to have been missed. It may be that female patients with dementia or cognitive decline would be more easily missed than males. Similarly of interest was the fact that all cases with psychosis (n=3) and parkinsonism (n=1) in our cohort happened to be male. Whether there is a true gender effect in the prevalence of dementia and other neuropsychiatric and extrapyramidal symptoms in C9-ALS warrants further research.

4.4 Conclusions

This study reinforces findings that C9-ALS has large variety in its phenotype and can present in any clinical form. Bulbar dysfunction was found to be relatively common, in keeping with previous studies. An earlier report of spinal-onset males having shorter survival was not seen in our smaller cohort, but spinal-onset males did have significantly more variance in their disease duration than other patients. In addition, we found that male patients had more FTD and cognitive decline than female patients, which should be assessed systematically in future studies. Similarly, further research on the possibility of any gender effect regarding neuropsychiatric symptoms is needed.

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